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09/830,026	10/20/2001	William D. Picking	UOK 5320.1	9340

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EXAMINER	
DEVI, SARVAMANGALA J N	

ART UNIT	PAPER NUMBER
1645	15

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No. 09/830,026	Applicant(s) PICKING ET AL.	
	Examiner S. Devi, Ph.D.	Art Unit 1645	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE ____ MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☐ Responsive to communication(s) filed on *20 August 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *See Continuation Sheet* is/are pending in the application.

4a) Of the above claim(s) *See Continuation Sheet* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *13-18, 21, 22, 25 and 101-103* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☒ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *20 October 2001* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. §§ 119 and 120

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

13)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application) since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

a) ☐ The translation of the foreign language provisional application has been received.

14)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121 since a specific reference was included in the first sentence of the specification or in an Application Data Sheet. 37 CFR 1.78.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s) *4*.
4) ☐ Interview Summary (PTO-413) Paper No(s). _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

Continuation of Disposition of Claims: Claims pending in the application are 1-7,10,12-18,21,22,25,26,29-33,38-44,47,51-66,70,72,73,77-80,82-87,90-92,94-96 and 98-103.
Continuation of Disposition of Claims: Claims withdrawn from consideration are 1-7,10,12,26,29-33,38-44,47,51-66,70,72,73,77-80,82-87,90-92,94-96 and 98-100.

DETAILED ACTION

Preliminary Amendments

1) Acknowledgment is made of Applicants' preliminary amendments filed 10/20/01 and 08/20/03 (paper no. 8 and 13). With this, Applicants have amended the specification.

Election

2) Acknowledgment is made of Applicants' election filed 05/01/03 (paper no. 10) in response to the written lack of unity mailed 04/01/03 (paper no. 9).

Applicants have elected invention III, claims 13-18, 21, 22, 25 and 101-103, with traverse. Applicants' traversal is on the grounds that the composition claimed in claim 1, i.e., a composition comprising a recombinant invasin protein of at least 95% purity, is the unifying special technical feature, and that neither Picking *et al.* nor Markart *et al.* teach the special technical feature. Applicants state that Markart *et al.* do not specifically indicate the degree of purity. Applicants contend that Picking *et al.* describe a method of purifying recombinant IpaB and IpaC of greater than 90% homogeneity, but not of at least 95% purity. Applicants submit that since invention groups III, IV and V are drawn to methods of producing and using the products of invention groups I, II and III, groups I-V should be rejoined. Applicants state that rejoinder of both inventions I and II does not impose a serious burden on the Office. Applicants contend that inventions I and II encompass claims 4, 31 and 54, however acknowledge that the two groups differ in regard to the amino acid sequence. With regard to the nucleotide sequences, Applicants cite MPEP 803.04 and state that normally ten sequences constitute a reasonable number for examination purposes and that up to ten independent and distinct nucleotide sequences will be examined in a single application without 'restriction'. Applicants assert that the examination of two amino acid sequences does not impose a serious burden on the Office.

Applicants' arguments have been carefully considered. As clearly set forth in the Office Action mailed 04/01/03 (paper no. 9), the various inventions claimed in the instant application were subject to a lack of unity, as opposed to a restriction requirement. The product claimed in claim 1 and a method of producing the product were disclosed in the art in 1996 by Oaks, Picking and Picking. *Clin. Diagnost. Lab. Immunol.* 3: 242-245, 1996 (see particularly Figure 1 legend). See the rejection(s) made below. The inventions lack unity since the special technical feature does not define

over the prior art. Furthermore, the products of inventions I and II do not share significant structural elements, and the methods of inventions IV-VIII do not share common steps and/or active compositions. With regard to search burden, a structural or sequence search performed on the product of invention I would not be co-extensive to the product of invention II, because of the lack of significant structural elements. It is important to note that the elected method of invention III is **not** related to the product claimed in claim 1, i.e., a purified recombinant invasin of at least 95% purity, because none of the claims grouped under invention III are drawn to a method of producing a recombinant invasin product of at least 95% purity. The previously cited Picking *et al.* and Markart *et al.* indeed taught the special technical feature of invention III, i.e., a method of producing a recombinant pure invasin protein. Furthermore, a product having 100% structural identity to the product claimed in claim 4 was also known in the art at the time of the invention. For instance, Kaniga *et al.* (*J. Bacteriol.* 177: 3965-3971, 1995 - Applicants' IDS) taught a protein having 100% sequence identity to the amino acid sequence of SEQ ID NO:1. Although the first claimed product of the invention and the first method of using the product or making the product is a permitted combination under PCT Rule 13.2, in the instant case, the special technical feature was already disclosed in the art as explained above, and therefore is not a unifying feature. Technically, the absence of a special technical feature would permit the separation of the method of using and the method of making the product from the product itself. Therefore, the lack of unity set forth in the Office Action mailed 04/01/03 is proper and is maintained.

Status of Claims

3) Claims 8, 9, 11, 19, 20, 23, 24, 27, 28, 34-37, 45, 46, 48-50, 57-60, 69, 71, 74-76, 81, 88, 89, 93 and 97 have been canceled via the preliminary amendment filed 04/20/01.

Claims 16, 17, 22, 26, 42-44, 61, 65, 68, 70, 72, 82, 90, 94, 98 and 100 have been amended via the preliminary amendment filed 04/20/01.

New claims 101-103 have been added via the preliminary amendment filed 04/20/01.

Claims 1-7, 10, 12-18, 21, 22, 25, 26, 29-33, 38-44, 47, 51-56, 70, 72, 73, 77-80, 82-87, 90-92, 94-96 and 98-103 are pending.

Claims 1-7, 10, 12, 26, 29-33, 38-44, 47, 51-56, 70, 72, 73, 77-80, 82-87, 90-92, 94-96 and 98-100 are withdrawn from consideration as being directed to non-elected inventions. See 37 C.F.R

1.142(b) and M.P.E.P § 821.03.

Elected claims 13-18, 21, 22, 25 and 101-103 are under examination. A First Action on the Merits is issued for these claims.

Sequence Listing

4) Acknowledgment is made of Applicants' submission of the raw sequence listing and CRF filed 08/20/03 (paper no. 13) which have been entered on 08/28/03 (paper no. 14).

Information Disclosure Statement

5) Acknowledgment is made of Applicants' information disclosure statement filed 08/27/01 (paper no. 4). The information referred to therein has been considered and a signed copy is attached to this Office Action (paper no. 15).

Priority

6) The instant application is a national stage 371 application of PCT/US99/24931, filed 10/21/1999 and claims priority to provisional applications 60/136,754 filed 06/01/1999 and 60/105,085 filed 10/21/1998 in United States.

Drawings

7) The drawings submitted in the instant application are not objected to by the Draftsperson under 37 C.F.R 1.84 or 1.152 and as such, the drawings have been approved as formal drawings.

Abstract

8) This application currently does not contain an abstract of the disclosure as required by 37 C.F.R 1.72(b). However, as this application is a 371 of application of PCT/US99/24931, a copy of the published abstract from this application is placed in the instant application as page number 59. If Applicants desired changes to the abstract, such changes should be directed to the abstract of the PCT/US99/24931.

Sequence Rule Non-Compliance

9) The instant specification, for example in Figure 1, recites one or more nucleotide sequences at least ten nucleotide base-long, yet does not identify the sequences with SEQ ID NO. as required under 37 C.F.R 1.821 through 1.825. Any sequences recited in the instant specification which are encompassed by the definitions for nucleotide and/or amino acid sequences as set forth in 37 C.F.R. 1.821(a)(1) and (a)(2) must comply with the requirements of 37 C.F.R 1.821 through 1.825. Note

that branched sequences are specifically excluded from this definition.

APPLICANT MUST COMPLY WITH THE SEQUENCE RULES WITHIN THE SAME TIME PERIOD AS IS GIVEN FOR RESPONSE TO THIS ACTION, 37 C.F.R 1.821 - 1.825. Failure to comply with these requirements will result in ABANDONMENT of the application under 37 C.F.R 1.821(g).

Specification - Informalities

10) The specification is objected to for the following reason:

The use of the trademark in the instant specification has been noted. For example, see page 33, last line: 'Sequanase'; line 16 on page 36: 'BioRad'; last line on page 44: 'Tween 20'; line 18 on page 27: 'Tween' and 'Triton'; lines 15 and 17 on page 35: 'HisBind'; line 15 on page 36: 'Coomassie ... Blue'. The recitation should be capitalized wherever they appear and be accompanied by the generic terminology. Each letter of the trademark must be capitalized. See M.P.E.P 608.01(V) and Appendix l. Although the use of trademark is permissible in patent applications, the propriety nature of the trademarks should be respected and every effort made to prevent their use in any manner which might adversely affect their validity as trademarks.

Rejection(s) under 35 U.S.C. § 112, Second Paragraph

11) Claims 13-18, 21, 22, 25 and 101-103 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite, for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

(a) Part b) of claim 13 has improper antecedence in the recitation: 'the combination of a)' because part a) of the claim does not recite any 'combination'. For proper antecedence, it is suggested that Applicants replace part b) of the claim with --transforming a host cell with the expression vector of a)--.

(b) Analogous criticism applies to claim 14.

(c) Claims 14 and 25 are vague and indefinite in the recitation: 'appropriate' expression vector. What structural and/or functional characteristics an expression vector has to have in order to qualify as an 'appropriate' expression vector is unclear.

(d) The term 'rapidly' in claims 13 and 14 is a relative term which renders the claims indefinite. The term 'rapidly' is not defined by the claims, the specification does not provide a

standard for ascertaining the requisite degree, and one of ordinary skill in the art would not be reasonably appraised of the scope of the invention.

(e) Claims 13, 14 and 25 are vague, indefinite and confusing in the recitation: 'conditions conductive to soluble protein expression', because it is unclear which 'soluble protein' Applicants are referring to. Is this soluble invasin protein or any generic soluble protein? If the former is intended, Applicants should consider replacing the recitation 'soluble protein' with --soluble invasin protein--.

(f) Claims 13, 14 and 25 are vague, indefinite and confusing in that the claims do not distinctly claim the subject matter which Applicants regard as the invention. Part d) of the claims include the limitation: 'extracting the protein from a host cell lysate, culture medium, or reconstituted organism'. Due to the absence of proper antecedence, i.e., --the host cell-- , the recitation encompasses 'a host cell' other than the one recited in part c) of the claims. It is unclear how 'the protein' in part d) of the claims can be extracted from a host cell other than the one recited in part c), and how 'the protein' can be extracted from any 'culture medium', or 'a reconstituted organism'. What is contained in the culture medium, and what organism is reconstituted, is unclear.

(g) Claims 13, 14 and 25 are vague, indefinite and confusing in the recitation: 'the method of said purification' (see part e). Part e) of the claims represents an affinity purification step within the claimed method. In order to distinctly claim the subject matter of the invention and for clarity, it is suggested that Applicants replace the limitation with --said affinity purification--.

(h) Claims 13, 14 and 25 are vague, indefinite and/or confusing in the limitation 'the denaturant' in part f) of the claims. The earlier parts of the claims recite 'a protein denaturant', but not any generic 'denaturant'. For proper antecedence and clarity, it is suggested that Applicants replace the limitation with --the protein denaturant--.

(i) Claims 13, 14 and 25 have improper antecedence in the limitation 'the protein solution' in part f) of the claims, because there is no earlier recitation of any 'protein solution' in the claims.

(j) Claims 13, 14 and 25 have improper antecedence in the limitation 'the purification process of e)' in part f) of the claims, because there is a recitation of --the affinity purification-- in part e) of the claims, but no earlier recitation of any 'purification process' in part e) of the claims.

(k) Part f) of claims 13, 14 and 25 are confusing in the recitation 'protein solubility',

because it is unclear the solubility of which protein does this refer to. If the recited solubility is of the invasin protein, Applicants should replace the recitation with --the solubility of the recombinant invasin protein--.

(l) Part g) of claims 13, 14 and 25 are confusing in the limitation: 'the purified protein', because it is unclear which 'protein' is encompassed in this limitation. The first line of the claims recite 'a purified recombinant invasin protein', but not any generic 'purified protein'.

(m) Analogous criticism applies to claims 22 and 103.

(n) In part g) of claims 13, 14 and 25, the scope of the limitation 'denaturant' in the 'denaturant-free solution' is not clear. It is not clear whether the limitation represents a protein denaturant or any generic denaturant.

(o) Claims 16 and 17 are vague and indefinite, because it is clear where does the antecedence come from for 'the' protein denaturant. Claims 16 and 17 depend from claim 14, which recites 'a protein denaturant' in part d) of the claim and 'a protein denaturant' in part e) of the claims.

(p) Analogous criticism applies to claims 101 and 102.

(q) Claim 21 is vague and indefinite in the recitation: "the step of removing the affinity purification moiety from the recombinant invasin protein". 'The recombinant invasin protein' containing the affinity purification moiety is available at the end of part e), part f) and part g) of claim 14, from which claim 21 depends. It is unclear whether the step recited in claim 21 is performed at the end of part e), part f) or part g) of claim 14.

(r) Claims 14 and 21 are vague and indefinite in the recitation 'moiety', because it is unclear what is contained in this limitation. The nature of the 'moiety' is not clear. Does this encompass a carbohydrate, a peptide, a protein, a lipid, or a glycoprotein? What characteristics a substance should have in order to qualify as a 'moiety' is unclear.

(s) Claims 15-18, 21, 22 and 101-103, which depend directly or indirectly from claim 13 or claim 14, are also rejected as being vague and indefinite because of the vagueness or indefiniteness identified above in the base claim.

Rejection(s) under 35 U.S.C § 102

12) The following is a quotation of the appropriate paragraphs of 35 U.S.C. § 102 that form the

basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

13) Claims 13-18, 21, 22 and 101-103 are rejected under 35 U.S.C. § 102(b) as being anticipated by Paul *et al.* (*Human Gene Therapy* 8: 1253-1262, 01 July 1997).

Paul *et al.* taught a method for the production of a purified recombinant soluble invasin protein which combines the use of a protein denaturant and affinity purification step. The method comprises inserting a polynucleotide encoding an invasin protein into an expression vector; transforming a host cell with the vector; growing the transformed host cell under conditions suitable for soluble invasin expression; obtaining the invasin protein from the host cell lysate with a solution comprising guanidine hydrochloride (i.e., a protein denaturant); performing an affinity purification of the invasin protein using Hexahis-tag in the presence of 8M urea (i.e., a protein denaturant); dialyzing the invasin protein solution using a buffer containing 6M urea (i.e., a protein denaturant); and diluting the purified protein into a buffer solution that does not contain urea. The purified recombinant soluble invasin protein was shown, via an internalization assay, to retain its biological functions. See entire document, especially 'Materials and Methods' on pages 1254 and 1255, and 'Results' on pages 1256 and 1257. With regard to the step of 'dilution' recited in claims 22 and 103, that such dilution in a denaturant-free solution takes place 'rapidly' within a minute in the prior art method, is inherent from the teachings of Paul *et al.* in light of the fact that the final recombinant invasin protein product remained soluble and biologically functional.

Claims 13-18, 21, 22 and 101-103 are anticipated by Paul *et al.*

14) Claims 13-16, 21, 22, 101 and 103 are rejected under 35 U.S.C. § 102(b) as being anticipated by Picking *et al.* (*Protein Expression and Purification* 8: 401-408, 1996 - Applicants' IDS).

Picking *et al.* taught a method for the production a purified recombinant invasin protein comprising inserting an *ipa* invasin gene DNA fused to a HisTag affinity moiety into a pET32b vector; transforming a host cell such as *E. coli* with the vector; growing the transformed *E. coli* under conditions suitable to express soluble invasin; extracting the recombinant invasin protein from sonicated bacterial lysate using a detergent, such as, Triton X-100; performing an affinity purification using HisTag followed by elution, dialysis (which further purifies and dilutes the preparation) in a

sodium phosphate-NaCl-glycerol buffer. The affinity purification moieties were removed by site-specific cleavage to obtain the purified recombinant invasin. The purified invasin did show immunological binding with specific monoclonal antibodies raised against natural forms of invasin proteins. See abstract; 'Materials and Methods'; 'Results'; Figures 1, 3 and 4; and 'Discussion'. That the prior art process of dialysis involves dilution of the purified invasin within a minute or less is inherent from the teachings of Picking *et al.* in light of the fact that the final recombinant invasin protein product remained immunologically functional.

Claims 13-16, 21, 22, 101 and 103 are anticipated by Picking *et al.*

15) Claims 13, 14, 16, 22, 101 and 103 are rejected under 35 U.S.C. § 102(b) as being anticipated by Leong *et al.* (*EMBO J.* 9: 1979-1989, 1990).

Leong *et al.* taught a method of producing a purified recombinant invasin protein comprising inserting a polynucleotide encoding an invasin protein into a plasmid expression vector; transforming an *E. coli* host cell with the vector; growing the host cell under suitable conditions; extracting the invasin protein from lysed host cells using a solution comprising 6M guanidine-HCl; and dialyzing the protein against a buffer containing a lesser concentration, i.e., 0.5M, guanidine-HCl followed by diluting the purified protein in a solution that does not contain guanidine-HCl. The method comprises the use of an affinity-chromatography purification step. The purified protein elicited antibodies. See entire publication, especially 'Materials and methods' on pages 1986 and 1987. That the prior art process of dialysis involves dilution of the purified invasin within a minute or less is inherent from the teachings of Leong *et al.* in light of the fact that the final recombinant invasin protein product remained biologically functional.

Claims 13, 14, 16, 22, 101 and 103 are anticipated by Leong *et al.*

Rejection(s) under 35 U.S.C § 103

16) The following is a quotation of 35 U.S.C. § 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person. having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham v. John Deere Co.*, 148 USPQ 459, that are applied

for establishing a background for determining obviousness under 35 U.S.C. § 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or unobviousness.

17) Claim 25 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Oaks *et al.* (*Clin. Diagnost. Lab. Immunol.* 3: 242-245, 1996) in view of Comb *et al.* (US 5,834,247) and/or Anilionis *et al.* (US 5,196,338), Thorne (US 5,552,294) and Seed (US 5,726,293).

The reference of Comb *et al.* is applied in this rejection because it qualifies as prior art under subsection (e) of 35 U.S.C § 102 and accordingly is not disqualified under U.S.C 103(a).

Oaks *et al.* taught a method for the production of a purified recombinant invasin protein comprising inserting a polynucleotide encoding an invasin protein and a six histidine affinity purification moiety into a pET expression vector; transforming an *E. coli* host cell with the vector; obtaining the invasin protein from a cytosolic extract of the host cell; and affinity purifying the protein using the six histidine residues, a nickel column and an elution solution containing 1M imidazole (i.e., protein denaturant). The purified IpaD invasin was diluted in PBS (i.e., a buffer that is denaturant-free). The affinity purified recombinant IpaD invasin protein was greater than 95% pure. See Figure 1; and pages 242 and 243.

Oaks *et al.* do not teach the step of using 6M urea to extract the protein, and are silent about the step of removal of the protein denaturant from the protein solution and the dilution being done in 10 seconds or less.

However, the use of urea at a preferred concentration range of 0.1M to about 15M for the release of a virulence-associated factor, such as, invasin, and in stabilization of its three dimensional concentration, was well known in the art at the time of the invention. For instance, Thorne disclosed the use of 0.1M to 15M urea in the release and stabilization of a virulence-associated invasin, including that of *Shigella* (see last paragraph in column 7; and paragraph bridging columns 3 and 4). The affinity purified recombinant IpaD invasin was used as a coating antigen in ELISA (see pages 243 and 244).

The specific use of 6M urea in the production and solubilization of bacterial membrane proteins or fusion proteins, and subsequent removal of urea from the extracted fusion protein by dialysis against a buffer that does not contain urea, was well known in the art at the time of the invention. For example, Anilionis *et al.* taught such a method (see third full paragraph in column 63).

Comb *et al.* taught the routine and conventional use of 6M urea in the solubilization and purification of recombinant fusion proteins or target proteins and the process of rapidly diluting the proteins to allow protein refolding. Comb *et al.* also taught that variation of different parameters including the urea concentration allows optimization of refolding efficiencies (see the first two paragraphs in column 50).

That the imidazole used in the prior art method qualifies as a protein denaturant is implicit from the teachings of Oaks *et al.* in light of what was well known in the art. For instance, Seed taught imidazole to be a mild denaturant (see line 48 in column 2).

Given the routine and conventional use of 6M urea in the production of recombinant target proteins or fusion proteins as taught by Comb *et al.* or Anilionis *et al.*, and given the disclosure by Thorne of the use of an urea concentration anywhere in the range of 0.1M to 15M specifically during the release and stabilization of virulence-associated invasin, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to add into Oak's method Comb's or Anilionis's step of treating invasin solution with 6M urea, and further adjust or optimize the time period within which to dilute the purified invasin in PBS to 10 seconds or less in Oaks' method, by routine optimizing experimentation, to produce the instant invention with a reasonable expectation of success. One of skill in the art would have been motivated to include the step of treatment with 6M urea into Oaks' method for the expected benefit of simultaneously releasing and stabilizing the invasin protein as taught by Thorne. The process of optimizing the time period within which to dilute the purified invasin in a denaturant-free PBS to an optimal time in an art-known process is well within the realm of routine experimentation and would have been obvious to a skilled artisan at the time of the instant invention. It has been held legally obvious and within the routine skill in the art to optimize a result-effected variable. In the instant case, the time period within which to dilute the purified invasin in a denaturant-free PBS is clearly a result-effected variable, and it

would have been obvious to vary or optimize the time to ten seconds or less by routine experimentation. One of skill in the art would have been motivated to rapidly dilute the purified protein as recited for the purpose of allowing proper protein refolding as taught by Comb *et al.*

Claim 25 is *prima facie* obvious over the prior art of record.

Relevant Prior Art

18) The prior art made of record and not relied upon in any of the rejections is considered pertinent to Applicants' disclosure:

- Rupp *et al.* (US 6,303,302) disclosed a method for the production of a purified recombinant invasin protein (see entire document).

- Marquart *et al.* (*Infect. Immun.* 64: 4182-4187, 1996 - Applicants' IDS) taught a method for the production of a purified recombinant invasin IpaC protein comprising inserting a polynucleotide encoding the invasin protein into a pET expression vector; transforming an *E. coli* host cell with the vector; culturing the transformed host cell under conditions suitable for soluble protein expression; extracting the recombinant invasin protein from a sonicated host cell lysate using a solution comprising Tris-HCl and imidazole; performing a nickel affinity purification using six histidine residues, followed by elution of the invasin protein in a solution comprising imidazole; and dialyzing (and therefore diluting) the protein against PBS buffer containing NaCl. The resultant protein did retain its immunological specificity to an invasin-specific antibody. See Figure 1; and 'Materials and Methods' on page 4183.

- Terajima *et al.* (*Microb. Pathogenesis* 27: 223-230, October, 1999) taught a method for the production of a purified recombinant soluble invasin Ipa protein comprising inserting a polynucleotide encoding the invasin protein into a expression vector; transforming a host cell with the vector; culturing the transformed host cell under conditions suitable for soluble protein expression; extracting the recombinant invasin protein from a sonicated host cell lysate using a solution comprising Tris-HCl and EGTA or EDTA; performing a maltose-resin affinity purification, followed by elution of the invasin protein; and diluting the protein against a buffer containing no sucrose. The resultant protein did retain its immunological specificity to an invasin-specific antibody. See 'Materials and Methods'.

- De Geyter *et al.* (*FEBS Lett.* 400: 149-154, 1997 - Applicants' IDS) identified the

need for providing urea during the isolation and purification of IpaC invasin protein. De Geyter *et al.* taught that in the absence of urea, IpaC is aggregated. De Geyter *et al.* used 4M urea. See 'Materials and Methods'; 'Results' and 'Discussion'.

- Marquart *et al.* (*Biochem. Biophys. Res. Commun.* 214: 963-970, 1995) taught a method of obtaining a purified soluble recombinant IpaD invasin by inserting the polynucleotide encoding the invasin fused with six histidine leader sequence into an expression vector; transforming an *E. coli* host cell with the vector; expressing the recombinant IpaD; affinity purifying the invasin using nickel resin; and removing the leader peptide with thrombin to obtain the recombinant invasin that remains soluble in aqueous solutions (see 'Materials and Methods' and page 968).

Remarks

19) Claims 13-18, 21, 22, 25 and 101-103 stand rejected.

20) Papers related to this application may be submitted to Group 1600, AU 1645 by facsimile transmission. Papers should be transmitted via the PTO Fax Center located in Crystal Mall 1 (CM1). The transmission of such papers by facsimile must conform with the notice published in the Official Gazette, 1096 OG 30, November 15, 1989. The CM1 facsimile center receives papers 24 hours a day and seven days a week. The RightFax number for submission of before-final amendments is (703) 872-9306. The RightFax number for submission of after-final amendments is (703) 872-9307.

21) Any inquiry concerning this communication or earlier communications from the Examiner should be directed to S. Devi, Ph.D., whose telephone number is (703) 308-9347. A message may be left on the Examiner's voice mail system. The Examiner can normally be reached on Monday to Friday from 7.15 a.m. to 4.15 p.m. except one day each bi-week which would be disclosed on the Examiner's voice mail system.

If attempts to reach the Examiner by telephone are unsuccessful, the Examiner's supervisor, Lynette Smith, can be reached on (703) 308-3909.

November, 2003

S. DEVI, PH.D.
PRIMARY EXAMINER